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Transglutaminase-induced corneal collagen cross-linking on the central cornea thickness and intraocular pressure in vivo

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Abstract

Background: Corneal collagen crosslinking (CXL) is a procedure for making bonds that connect polymer chains to one another. Corneal CXL aims to slow or stop the progression of keratoconus by using photooxidative therapy so as to increase stromal rigidity. Transglutaminase enzymes are currently widely used in the food industry. Recent studies have shown that mRNA, fibronectin, and transglutaminase were found to be more abundant in human corneal keratocytes treated with UVA and riboflavin. Transglutaminase is considered to reduce discomfort caused by UVA irradiation.

Methods: A total of 21 white New Zealand rabbits were divided into three groups, namely, transglutaminase-induced CXL group, epithelial-off CXL group, and transepithelial CXL group. The ocular surface was treated with a 1 U/mL microbial transglutaminase solution, and both the epithelial-off and transepithelial groups were exposed to clinical ultraviolet A-riboflavin (UVA/RF). The efficacy of each group was evaluated on the 14^{th} day after the procedures. The central corneal thickness (CCT) and intraocular pressure (IOP) were evaluated using Corneal Visualization Scheimpflug Technology (Corvis ST).

Results: The transglutaminase-induced CXL group exhibited the highest mean CCT (370.14 ± 38.85) in comparison with the UVA/RF epithelial-off group (368.00 ± 25.48) and the UVA/RF transepithelial group (369.86 ± 23.43). The transglutaminase-induced CXL group had the highest IOP mean (8.50 ± 3.02) compared with the UVA/RF epithelial-off (6.50 ± 3.07) and UVA/RF transepithelial groups (7.00 ± 1.90). There were no significant differences in CCT (p = 0.990) or IOP (p = 0.563) between the groups.

Conclusions: The findings of this study suggest that there are no significant differences between the transglutaminase-induced CXL group and the UVA/RF CXL group. The safety of transglutaminase-induced CXL could be comparable to that of UVA/RF CXL in terms of altering CCT and IOP, which are two factors contributing to corneal rigidity

Troubleshooting

Subject and measures

1 Ethical approval was obtained from the Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KEH.071.07.2022). All procedures were performed to ethical standards.

2 Twenty-one normal New Zealand white rabbits were enrolled, and the right eye was treated. All the rabbits were divided into three groups, namely, transglutaminase-induced CXL group, epithelial-off CXL group, and transepithelial CXL group. There were seven rabbits in each group and we used random allocation for the randomization.

3 All rabbits were healthy male, between two and three months of age, and weighed between 3 and 4 kgAll rabbits were provided by the Faculty of Veterinary Medicine, Universitas Airlangga. The experiments were performed at Veterinary Hospital Universitas Airlangga.

4 The equipment utilized in this study includes individual rabbit cages with ideal room temperature (24°C), enough ventilation, and good ad libitum. The portions of food and drink are the same for all rabbits.

Cross-linking procedure

5 Anesthesia was induced via intramuscular injection of 0.1 mL/kgBW xylazine 2% (Xyla, Interchemie, Netherland) and 6–10 mg/kgBW ketamine (Ket-A-100®, Agrovet Market s.a. Lima, Peru).

6 The corneal epithelium of the first group was removed using an 8 mm MacRae Photorefractive Keratectomy (PRK) well and alcohol 20% for 40 seconds, then the loose epithelium was removed with a hockey knife. After the epithelial-off procedure, transglutaminase solution 1U/mL (Zedira, Germany) was dripped onto the cornea every two minutes for 30 minutes, for a total of 16 times.

7 The cornea epithelial of the transepithelial group was not removed, then the riboflavin transepithelial (Peschke TE, Peschke Meditrade GmbH, Switzerland) was dripped onto the cornea every two minutes for 30 minutes. Subsequently, UVA exposure (365 ± 5 nm, 3 mW/cm^2, 8-mm diameter light spot) was performed using a UVA lamp CCL-Vario System (Peschke Trade GmbH, Switzerland) while continuously dripping the riboflavin onto the cornea every two minutes for 30 minutes.

8 The cornea epithelial of the epithelial-off group was removed with the same procedure, then the riboflavin epithelial-off (Ribolink, Aurolab, India) was dripped two times onto the cornea. Subsequently, UVA exposure was performed using the same technique while continuing to drip the riboflavin onto the cornea every five minutes for 30 minutes.[12,13]

9 Immediately following the procedure, an eye ointment containing chloramphenicol (Cendo, Indonesia) was used. The ointment was administered every eight hours for fourteen days.

CCT and IOP evaluation procedure

10 Fourteen days after treatment, the CCT and IOP of the rabbits were evaluated using Corvis ST (Oculus Optikgeräte GmbH, Wetzlar, Germany). Before the procedure, the rabbits were first anesthetized using 0.15 mL/kg xylazine and 6–10 mg/kg ketamine intramuscularly. All examinations were conducted by the same experienced ophthalmology technician to remove potential interobserver variability.

11 The heads of the rabbits were positioned in front of the instrument to ensure that the cornea was 11 mm away from the air nozzle. In the instance of improper imaging or alignment concerns, a retake was performed in order to meet quality standards. The air puff was released and the ultrafast camera captured 140 horizontal cross-sectional corneal images; subsequently, CCT and IOP were determined.

Statistical analysis

12 Comparative between data were tested using the one-way ANOVA followed with a *post hoc*test if the data were normally distributed. If the data were not normally distributed, they would be analyzed using the Kruskal–Wallis test and continued by using the Mann–Whitney test. The result is considered significant if the p-value is < 0.05. All statistical data were processed using SPSS 26.0 software (RRID:SCR_002865) (SPSS, Chicago. IL, USA).